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RESEARCH PAPER

Effect of pre-soaking and pre-chilling treatments on seed germination of *Pinus roxburghii* provenances from western Himalaya, India

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Abstract: After subjecting the seeds to GA_3 and H_2O_2 treatments for 24 h and chilling at 2–3°C for a period of 15 days, we conducted the seed germination tests for 21 different seed sources of *Pinus roxburghii* from western-central Himalaya under laboratory conditions at various temperatures *viz*., 20°C, 25°C and 30°C inside a seed germinator. The results reveal that the soaking of seeds in H_2O_2 (1% v/v) and GA_3 (10 mg·L^{-1}) solutions manifested 82.39% and 78.19% germination, respectively whereas untreated seeds exhibited 70.79% average germination. Both GA_3 and H_2O_2 treatments caused an appreciable shortening of the germination period by 8 days and 10 days, respectively. Moist-chilling did improve the rate and percentage of germination when germinated at 20°C over 21 days; however total germination was not affected at temperatures 25ºC and 30°C. Although the seeds of *P. roxburghii* germinate well due to lack of dormancy, the increasing demand for large quantities of seeds of *P. roxburghii* for reforestation programmes make pre-sowing treatments useful in improving the rate and percentage of germination.

Keywords: seed germination; seed sources; provenances; temperature; GA_3; H_2O_2; chilling; chir pine; *Pinus roxburghii*; Himalaya

Introduction

Pinus roxburghii Sargent, commonly known as the chir pine or Himalayan long needle pine is an indigenous, fire resistant tree species of western-central Himalaya, which occurs naturally between 450 m to 2 300 m elevation (Sharma and Baduni 2000). This species constitutes about one-third of the total forest area of Uttarakhand Himalaya, India (Tewari 1994). Qualities such as a straight cylindrical bole, rapid growth, high volume returns, yielding of timber and resin, and capacity to colonize in degraded habitats make this species a precious resource of Himalayan region., The *P. roxburghii* is being planted on a massive scale in different parts of the country as a source of wealth due to its multifarious uses particularly the resin of commercial value. Anemophily (Khanduri and Sharma 2007) and wide geographic distribution of this species manifested considerable natural variation. To explore this variability, provenance testing is essential to obtain quality traits for afforestation programmes and genetic improvement of specific traits.

Selection of the best provenances of the desired species for a given site or region is necessary for achieving maximum productivity. In those species for which there are data on individual tree variability, genetic differences are associated with place of origin, which have often been several times as great as those among individual trees in the same stand. Plus trees chosen for selective breeding, without regard to provenance performance might give birth to an inferior race. Therefore, it is particularly necessary to do provenance testing prior to more intensive breeding work.

Temperature affects the percentage and rate of germination (Baskin and Baskin 1992; Landis et al. 1998) through its effects on seed deterioration, loss of dormancy (Wang and Berjak 2000) and the germination process itself (Roberts 1988; Bewley and Black 1994; Ghildiyal and Sharma 2005). Some chemicals, like hydrogen peroxide and other growth promoting substances such as gibberellic acid have been found helpful in enhancing germination rate of the seeds in many species (Vogt 1970; Krishnamurthy 1973; Chandra and Chauhan 1976; Shafiq 1980; Thapliyal et al. 1985; and Ghildiyal 2003), through which losses in seed germination could be minimised (Quarberg and Jahns 2000). Cold moist stratification is a common practice to enhance the rate and percentage of germination of dormant seeds of most conifers (Mergen 1963; Fowler and Dwight 1964; Roberts and Ellis 1982; Wang and Berjak 2000; Rawat et al. 2008). The effective temperatures for cold stratification are from about 0ºC to 10ºC, but 5ºC being optimal for many species (Stokes 1965; Baskin and Baskin 2001). Keeping in view the all above facts in

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mind, the present study was aimed at understanding the efficacy of hydrogen peroxide, gibberellic acid, and moist chilling treatments on the germination of seeds of different provenances of *P. roxburghii*.

Materials and methods

Mature seeds of *P. roxburghii* were collected during the months of February – April, 2000 from 21 different seed sources of two states, i.e. Uttarakhand (U.K.) and Himachal Pradesh (H.P.), situated in the Western-Central Himalaya, India. The details of the seed sources/study areas are presented in Table 1& Fig. 1.

The studies pertaining to seed germination after pre-soaking (at room temperature, 25°C) and pre-chilling (3–5°C) treatments were carried out at various temperatures *viz*., 20°C, 25°C and 30°C inside a seed germinator (Model No. 8LT-SGL CALTAN). The seeds of all the provenances of *P. roxburghii* were germinated at similar specified temperatures after applying following treatments to each set:

Treatment 1: Soaking of the seeds in distilled water at room temperature (25°C) for 24 h.

Treatment 2: Soaking of the seeds in Gibberellic acid (GA_3 10 mg/L) at room temperature (25°C) for 24 h.

Treatment 3: Soaking of the seeds in Hydrogen peroxide (H_2O_2 1% v/v) at room temperature (25°C) for 24 h.

Treatment 4: Seeds treated with Gibberellic acid (GA_3 10 mg/L) as above at room temperature (25°C) for 24 h and then chilled for 15 days at 3–5°C.

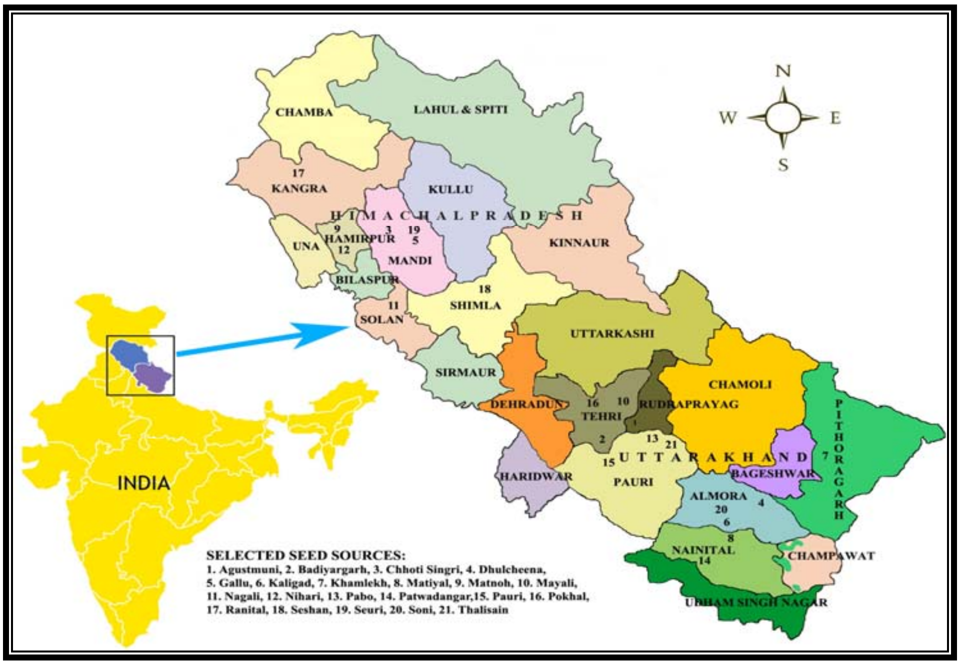

Fig. 1 Location Map of the Study Area.

The seeds of different provenances having same level of ripeness were collected and subjected to viability test by floating method to select the viable seeds. For germination, the seeds in five replicates of 100 seeds each were placed in Petri dishes (diameter- 10 cm) containing two filter papers, kept in the germinator, and maintained at desired temperature. Observations were recorded daily regarding germinated /non-germinated seeds up to 21 days. Radicle emergence was taken as the criteria for germination.

Chilling of seeds was carried out in folded-over-polythene bags at 3–5°C for a period of 15 days (as per method suggested by Tompsett and Pritchard 1998), after soaking the seeds in 10 mg/L Gibberellic acid for 24 h followed by brief drying. The chilled seeds were then subjected to germination tests in all specified temperatures.

Percent germination is the ratio of seeds germinated at the completion of the germination period, whereas, germination value is an index, combining both speed and completeness of germination; which according to Czabator (1962) can be expressed as:

$$GV = PV \times MDG$$

where, *GV* is the germination value, *PV* is the peak value of germination, and MDG is the mean daily germination

The statistical analysis of each parameter was carried out on mean values and the analysis of variance (ANOVA) was performed using SPSS package. The critical difference (CD) was calculated as:

$$CD = SEd \times t_{0.01}$$

Where, *SEd* is the standard error of difference calculated as SEd = $\sqrt{(2 \times Me/r)}$, Me = Mean sum of square due to error, r = Number of replicates. Pearson correlation was calculated between germination parameters and geographic factors (latitude, longitude, altitude and rainfall) to rectify the effect of geographic factor on seed germination.

Table 1. Geographic and climatic descriptions of the selected seed sources of *Pinus roxburghii*

Provenance	District (State)	Latitude (N)	Longitude (E)	Altitude (m)	Temperature (°C)		Mean Annual rainfall (mm)
					Min.	Max.	
Agustmuni	Rudraprayag (U.K.)	30° 23'	79° 02'	875	4.31	36.59	833
Badiyargarh	Tehri (U.K.)	30° 17'	78° 50'	1080	7.5	36.3	930
Chhoti Singri	Mandi (H. P.)	31° 49'	76° 59'	1220	0.3	32.5	1025
Dhulcheena	Almora (U.K.)	29° 42'	79° 49'	1850	-0.14	26.4	1125
Gallu	Mandi (H. P.)	31° 42'	77° 01'	1520	-0.2	31.4	1100
Kaligad	Almora (U.K.)	29° 38'	79° 25'	1800	0.42	26.86	1060
Khamlekh	Pithoragarh (U.K.)	29° 47'	80° 04'	1450	3.1	31.2	1230
Matiyal	Nainital (U.K.)	38° 10'	69° 20'	1740	3.8	23.4	2270
Matnoh	Hamirpur (H. P.)	31° 45'	76° 43'	980	0.8	33.6	1150
Mayali	Tehri (U.K.)	30° 23'	78° 47'	1400	2.6	25.1	1030
Nagali	Solan (H. P.)	30° 54'	77° 12'	1545	0.5	34.1	1000
Nihari	Hamirpur (H. P.)	31° 29'	76° 28'	800	1.2	35.4	1125
Pabo	Pauri (U.K.)	30° 15'	79° 01'	1640	1.8	32.4	875
Patwadangar	Nainital (U.K.)	29° 16'	79° 20'	1500	7.4	28.5	2850
Pauri	Pauri (U.K.)	30° 09'	78° 48'	1660	-0.48	26.3	1792
Pokhal	Tehri (U.K.)	30° 25'	78° 59'	820	5.7	37.63	800
Ranital	Kangra (H. P.)	32° 10'	76° 05'	960	0.2	32.5	1350
Seshan	Shimla (H. P.)	31° 07'	77° 45'	1540	0.5	30.3	1075
Seuri	Mandi (H. P.)	31° 50'	77° 02'	1460	0.15	33.2	925
Soni	Almora (U.K.)	29° 12'	79° 24'	1650	2.3	28	1040
Thalisain	Pauri (U.K.)	30° 02'	79° 03'	1640	1.9	31	1025

* U. K.= Uttarakhand, H. P.= Himanchal Pradesh

Results

Germination of seeds of various provenances after pre-soaking treatments (H_2O_2 1% v/v and $GA_3$10 mg/litre) under different temperature regimes, (i.e., 20°C, 25°C and 30°C) have yielded significant differences in seed germination. The data analysed for its variance indicated much variation between different seed sources, which are presented in Table 2. The detailed treatment-temperature interactions are given below.

Soaking of seeds in distilled water (as control)

At 20°C the maximum germination percentage was recorded for Pokhal provenance (98.0%±2.00%) and minimum for Chhoti Singri provenance (46.0%±4.64%) (Table 2). However, the maximum germination value was recorded for Mayali provenance (18.74±1.83), and minimum for Chhoti Singri provenance (0.73±1.23). On the other hand, at 25°C, the maximum germination percentage was recorded for Dhulcheena provenance (97.8%±0.66%), and minimum for Chhoti Singri provenance (33.2%±1.10%). The higher germination value was recorded for Mayali provenance (24.43±1.99); whereas, the lowest germination value was again recorded for Chhoti Singri provenance (0.59±0.17). At 30°C, Dhaulcheena provenance showed maximum germination percentage (86.5% ±0.87%) and minimum was recorded for Kaligad provenance (20.6% ±1.97%).

Soaking of seeds in GA_3

Seeds treated with gibberellic acid reflected lesser germination percentage as compared to seeds treated with H_2O_2 1% v/v. Substantial germination after GA_3 treatment was recorded in Pokhal (94.0%±2.45%), Dhulcheena (92.6%±1.78%), Kaligad (88.2%±3.25%), Soni (86.6%±4.46%) and Thalisain (86.0%±6.00%) provenances, whereas minimum germination was recorded for Chhoti Singri provenance (56.2%±3.92%). Remarkable germination values were observed for Mayali (16.27±0.36), Soni (12.56±1.48) and Agustmuni (12.16±0.48) provenances, as compared to the value recorded for Chhoti Singri provenance (1.94±1.54). At 25°C, higher germination percentages were recorded for Dhulcheena (98.4%±1.75%), Nagali (97.6%±1.47%), Seshan (96.8%±1.86%), Matnoh (93.6%±0.75%) and Kaligad (93.2%±1.60%) provenances and lowest germination percentage was recorded for Khamlekh provenance (60.8%±1.86%). It is interesting to point out that the seeds treated with gibberellic acid have shown better germination percentage at 30°C as compared to 20°C and 25°C. The provenances that showed better germination percentages at 30°c were Matiyal (97.2%±0.86%) and Pokhal (94.0%±1.23%). Poor germination was recorded for Nihari (45.9%±1.51%) and Chhoti Singri (46.1%±1.65%) provenances (Table 2). It was also observed during the experiment that on an average this treatment reduced the germination period by eight days in all the temperature regimes.

Soaking of seeds in H_2O_2 1% v/v

At 20°C, the highest and lowest germination percentages were recorded for Pokhal (98.0%±2.00%), and Chhotisingri provenances (68.4% ±2.72%), respectively. Similarly the highest and lowest germination values were recorded for Mayali provenance (21.66 ±1.62), and Chhoti Singri provenances (2.76±0.62), respectively. At 25°C, higher germination percentages were recorded for Soni (98.4%±0.85%), Patwadangar (98.0%±0.63%),

Thalisain (96.0%±0.90%), Matiyal (94.8% ±1.10%), Nihari (93.6 ±2.17) and Matnoh (93.6 ±1.17%) provenances. The seeds from Chhoti Singri provenance had the lowest germination percentage (34.4%±1.33%). The seeds of all provenances can be germinated at 30°C, and some of the provenances i.e., Seuri (96.8 ±1.46%), Matnoh (96.2 ±1.53%), Thalisain (96.2 ±0.86%), and Agustmuni (93.6 ±1.44%), have reflected higher germination percentages, whereas Chhoti Singri (26.5 ±0.87%) and Nihari (46.6 ±1.19%) provenances have shown lower germination percentages (Table 2). Further, the experiment revealed that this treatment shortened the germination period in all the aforesaid temperatures averagely by 10 days.

Table 2. Effect of different treatments and temperatures on seed germination percentage and germination value of various provenances of *Pinus roxburghii*

Provenance	Seed germination percentage (data in standardized form) and germination value (data in italics form)								
	20°C			25°C			30°C		
	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)
Agustmuni	78.4 ±2.62	88.2 ±2.54	85.2 ±2.10	75.2 ±1.02	83.2 ±1.02	81.2 ±1.10	63.4 ±1.08	93.6 ±1.44	87.8 ±1.50
	16.32 ±1.42	*12.72 ±0.83*	*12.16 ±0.48*	*18.39 ±2.57*	*14.97 ±0.94*	*14.08 ±1.41*	*7.09 ±1.28*	*21.24 ±0.97*	*14.79 ±0.97*
Badiyargarh	52.2 ±1.58	78.2 ±2.42	69.6 ±5.62	39.2 ±1.36	83.6 ±1.33	61.2 ±1.10	51.6 ±1.21	64.2 ±1.72	81.0 ±1.18
	4.62 ±1.76	*12.68 ±1.42*	*2.18 ±0.32*	*2.84 ±0.74*	*17.85 ±2.33*	*6.38 ±1.13*	*1.59 ±0.36*	*2.67 ±0.55*	*4.47 ±0.96*
Chhoti Singri	46.0±4.64	68.4 ±2.72	56.2 ±3.92	33.2±1.10	34.4 ±1.33	73.2 ±1.20	32.9 ±0.91	26.5 ±0.87	46.1 ±1.65
	0.73 ±1.23	*2.76 ±0.62*	*1.94 ±1.54*	*0.59 ±0.17*	*0.88 ±0.10*	*3.72 ±1.57*	*0.24 ±0.01*	*0.13 ±0.01*	*0.48 ±0.04*
Dhulcheena	90.0 ±1.48	86.2 ±0.73	92.6 ±1.78	97.8 ±0.66	86.6 ±0.81	98.4 ±1.75	86.5 ±0.87	92.7 ±1.17	65.5 ±1.31
	4.68 ±1.32	*10.24 ±0.83*	*11.39 ±1.73*	*5.74 ±0.38*	*10.03 ±0.93*	*11.14 ±1.41*	*3.08 ±0.59*	*7.49 ±0.46*	*1.70 ±0.38*
Gallu	75.6 ±3.55	78.4 ±3.34	72.6 ±6.65	86.0 ±0.71	73.2 ±1.10	66.0 ±1.30	65.7 ±1.46	86.3 ±1.66	65.9 ±1.41
	6.34 ±0.92	*9.47 ±0.93*	*7.25 ±0.89*	*7.81 ±0.86*	*7.02 ±1.06*	*6.19 ±0.66*	*1.96 ±0.37*	*4.44 ±0.69*	*2.66 ±0.47*
Kaligad	82.2 ±1.26	90.6 ±2 .38	88.2 ±3.25	80.4 ±0.75	93.2 ±1.10	93.2 ±1.60	20.6 ±1.97	72.3 ±1.67	73.1 ±1.85
	5.14 ±1.67	*8.59 ±1.44*	*6.67 ±0.96*	*5.09 ±0.70*	*16.70 ±1.17*	*5.98 ±0.98*	*0.22 ±0.02*	*3.88 ±0.87*	*1.83 ±0.34*
Khamlekh	60.4 ±1.55	84.2 ±0.57	75.4 ±0.85	53.2 ±1.10	85.8 ±0.58	60.8 ±1.86	53.2 ±1.14	79.6 ±1.72	52.1 ±1.32
	3.52 ±1.52	*9.42 ±0.64*	*4.23 ±0.36*	*2.29 ±0.88*	*6.19 ±1.42*	*1.80 ±0.67*	*1.44 ±0.24*	*4.80 ±0.47*	*1.67 ±0.32*
Matiyal	64.2 ±1.34	87.2±0.92	83.2 ±1.93	60.0 ±1.42	94.8 ±1.10	85.8 ±1.36	84.9 ±1.71	65.5 ±1.52	97.2±0.86
	2.29 ±1.74	*6.61 ±1.24*	*3.07 ±1.55*	*2.85 ±0.64*	*8.89 ±1.42*	*5.25 ±1.45*	*6.35 ±0.40*	*5.42 ±0.57*	*9.16 ±0.96*
Matnoh	84.0±2.72	90.2 ±1.48	87.4 ±2.74	80.0 ±1.42	93.6 ±1.17	93.6 ±0.75	53.4 ±1.12	96.2 ±1.53	79.4 ±2.02
	8.27 ±0.76	*6.93 ±0.70*	*4.19 ±0.52*	*6.29 ±1.26*	*11.27 ±0.61*	*5.38 ±1.49*	*0.72 ±0.05*	*2.18 ±0.50*	*1.74 ±0.08*
Mayali	76.2±4.23	80.4 ±4.16	72.2 ±5.27	81.6 ±1.17	88.0 ±0.90	79.6 ±1.17	63.8 ±1.50	62.2 ±1.50	64.2 ±1.99
	18.74 ±1.83	*21.66 ±1.62*	*16.27 ±0.36*	*24.43 ±1.99*	*66.62 ±2.87*	*43.81 ±1.42*	*6.99 ±0.90*	*7.41 ±0.39*	*4.64 ±0.82*
Nagali	76.2 ±5.51	75.4 ±4.63	79.4 ±5.56	80.4 ±0.95	79.6 ±0.75	97.6 ±1.47	76.2 ±1.77	80.6 ±1.86	85.8 ±1.56
	8.96 ±0.37	*6.16 ±0.43*	*9.36 ±2.19*	*4.33 ±1.23*	*12.63 ±0.86*	*10.95 ±1.29*	*2.29 ±0.38*	*6.33 ±1.07*	*8.81 ±1.22*
Nihari	62.4 ±4.12	80.6 ±0.97	74.8 ±1.21	66.6 ±0.93	93.6 ±2.17	85.4 ±1.66	26.7 ±0.93	46.6 ±1.19	45.9 ±1.51
	4.21 ±1.42	*5.68 ±1.80*	*8.71 ±1.46*	*3.46 ±0.96*	*9.12 ±0.50*	*5.14 ±1.23*	*0.39 ±0.04*	*0.90 ±0.04*	*0.28 ±0.03*
Pabo	86.4 ±3.67	90.6 ±4.35	72.4 ±7.28	84.0 ±2.42	85.2 ±1.02	65.2 ±1.50	75.2 ±1.43	84.2 ±2.20	81.4 ±1.36
	7.25 ±0.84	*9.21 ±2.67*	*5.92 ±1.42*	*5.49 ±0.94*	*24.38 ±1.69*	*7.21 ±1.54*	*17.98 ±2.00*	*13.60 ±0.93*	*13.93 ±1.09*
Patwadangar	84.6 ±2.14	94.0 ±1.23	70.0 ±2.82	86.6 ±0.51	98.0 ±0.63	66.0 ±1.82	66.3 ±1.01	86.7 ±1.28	81.0 ±1.73
	7.23 ±0.48	*11.75 ±1.86*	*4.24 ±1.64*	*6.04 ±1.69*	*17.44 ±1.19*	*3.36 ±1.07*	*1.80 ±0.41*	*5.26 ±0.79*	*1.95 ±0.38*
Pauri	82.2 ±1.92	80.8 ±3.56	82.6 ±2.16	85.6 ±1.17	90.4 ±1.17	90.0 ±1.62	55.2 ±1.86	65.4 ±1.78	58.0 ±1.64
	8.68 ±1.32	*11.33 ±1.86*	*10.71 ±0.77*	*4.81 ±1.65*	*32.19 ±2.35*	*17.46 ±1.17*	*3.53 ±0.99*	*10.76 ±0.83*	*2.30 ±0.56*
Pokhal	98.0 ±2.00	98.0 ±2.00	94.0 ±2.45	82.2 ±2.06	88.4 ±0.75	65.2 ±1.40	83.2 ±1.86	80.2 ±1.80	94.0 ±1.23
	3.91 ±0.53	*8.91 ±0.73*	*3.43 ± 0.32*	*10.88 ±1.18*	*18.29 ±1.61*	*5.29 ±1.36*	*6.03 ±0.88*	*11.12 ±0.37*	*7.18 ±0.73*
Ranital	68.2 ±6.28	83.0 ±2.72	75.0 ±4.32	73.2 ±1.16	86.2 ±0.86	73.6 ±0.75	40.9 ±1.62	73.1 ±1.79	72.9 ±1.50
	3.88 ±1.78	*7.25 ±2.07*	*2.63 ±1.21*	*5.75 ±1.81*	*12.48 ±0.68*	*4.03 ±1.33*	*0.33 ±0.06*	*3.70 ±0.86*	*0.25 ±0.02*
Seshan	70.8 ±2.39	78.0 ±1.86	80.2 ±3.84	73.2 ±1.10	92.2 ±1.28	96.8 ±1.86	70.4 ±1.44	83.8 ±2.06	82.2 ±1.72
	6.43 ±0.79	*7.85 ±0.81*	*5.54 ±1.70*	*6.51 ±1.36*	*9.36 ±0.70*	*9.32 ±1.33*	*2.21 ±0.45*	*4.86 ±1.01*	*6.35 ±0.55*
Seuri	80.2 ±7.36	84.0 ±0.65	80.0 ±8.39	87.0 ±1.00	80.0 ±2.42	85.8 ±0.68	73.1 ±0.86	96.8 ±1.46	86.4 ±0.82
	5.86 ±0.54	*8.32 ±1.28*	*6.26 ±0.79*	*4.83 ±0.68*	*8.12 ±0.84*	*5.24 ±0.86*	*1.04 ±0.15*	*2.98 ±0.55*	*1.85 ±0.07*
Soni	86.2 ±0.86	97.4 ±1.06	86.6 ±4.46	86.6 ±0.93	98.4 ±0.85	86.2 ±1.69	60.1 ±1.46	71.7 ±1.55	85.2 ±1.37
	8.76 ±0.93	*14.37 ±1.32*	*12.56 ±1.48*	*8.12 ±1.16*	*17.06 ±0.93*	*12.36 ±2.15*	*2.63 ±0.54*	*4.62 ±0.60*	*5.05 ±1.85*
Thalisain	90.0 ±5.48	94.0 ±4.60	86.0 ±6.00	90.0 ±1.42	96.0 ±0.90	83.2 ±1.10	80.0 ±1.92	96.2 ±0.86	89.4 ±1.36
	2.74 ±0.53	*7.83 ±1.31*	*3.79 ±0.60*	*7.26 ±0.86*	*43.57 ±1.95*	*6.10 ±1.55*	*3.33 ±1.02*	*40.66 ±1.51*	*5.32 ±0.89*
Mean	75.92	84.86	79.22	75.33	85.92	80.38	61.11	76.40	74.98
	6.60	*9.51*	*6.79*	*6.85*	*17.38*	*9.06*	*3.39*	*7.83*	*4.59*
Range	46.0-98.0	68.4-98.0	56.2-94.0	33.2-97.8	34.4-98.4	60.8-98.4	20.6-86.5	26.5-96.8	45.9-97.2
	0.73-18.74	*2.76-21.66*	*1.94-16.27*	*0.59-24.43*	*0.88-66.62*	*1.80-43.81*	*0.22-17.98*	*0.13-40.66*	*0.25-14.79*
C.D at 1%	5.44	4.16	6.12	5.76	4.24	3.72	5.81	6.19	7.23
	2.28	*6.1*	*8.17*	*4.67*	*8.89*	*4.58*	*1.18*	*3.86*	*3.51*

Pre-chilling effects on germination

It has now been well established that presowing treatments of seeds by cold stratification enhances the rate and percentage of germination of seeds of many tree species. Therefore, we compared the germination of pre-chilled and unchilled seeds of various provenances (Table 3). Great similarity in seed germination percentages were recorded between pre-chilled and unchilled seeds. Nevertheless, germination values were recorded higher in unchilled seeds as compared to the pre-chilled seeds. A perusal

of Table 3 further revealed that the number of seed sources, particularly Agustmuni, Pabo, Gallu, Ranital and Nihari, excelled over other seed sources.

Table 3. Seed source variations, mean germination percentages (data in standardized form) and Germination values (data in italics form) of pre-chilled and unchilled seeds of *Pinus roxburghii* at different temperatures

Provenance	Pre-chilled			Unchilled		
	20°c	25°c	30°c	20°c	25°c	30°c
Agustmuni	86.2 ±4.94	84.5 ±0.91	79.2 ±4.64	85.2 ±2.10	81.2 ±1.10	87.8 ±1.50
	8.38 ±1.12	*8.76 ±1.70*	10.28 ±0.92	12.16 ±0.48	14.08 ±1.41	14.79 ±0.97
Badiyargarh	73.4 ±2.19	67.0 ±1.25	72.4 ±4.23	69.6 ±5.62	61.2 ±1.10	81.0 ±1.18
	2.27 ±0.68	*5.37 ±1.43*	*6.25 ±0.84*	*2.18 ±0.32*	*6.38 ±1.13*	*4.47 ±0.96*
Chhoti Singri	62.0 ±4.26	78.4 ±0.86	51.3 ±2.56	56.2 ±3.92	73.2 ±1.20	46.1 ±1.65
	1.67 ±2.42	*5.77 ±1.39*	*2.14 ±0.36*	*1.94 ±1.54*	*3.72 ±1.57*	*0.48 ±0.04*
Dhulcheena	94.6 ±2.56	98.8 ±0.92	63.2 ±3.24	92.6 ±1.78	98.4 ±1.75	65.5 ±1.31
	9.32 ±1.84	*9.75 ±1.87*	*2.06 ±0.54*	11.39 ±1.73	11.14 ±1.41	*1.70 ±0.38*
Gallu	78.6 ±8.23	70.4 ±1.48	68.4 ±2.83	72.6 ±6.65	66.0 ±1.30	65.9 ±1.41
	8.16 ±1.25	*3.72 ±0.75*	*4.64 ±1.37*	*7.25 ±0.89*	*6.19 ±0.66*	*2.66 ±0.47*
Kaligad	90.2 ±3.68	94.5 ±1.36	75.6 ±2.48	88.2 ±3.25	93.2 ±1.60	73.1 ±1.85
	6.44 ±1.12	*4.63 ±0.74*	*2.35 ±1.07*	*6.67 ±0.96*	*5.98 ±0.98*	*1.83 ±0.34*
Khamlekh	83.2 ±4.63	66.0 ±1.24	58.2 ±2.52	75.4 ±0.85	60.8 ±1.86	52.1 ±1.32
	4.39 ±0.67	*2.47 ±0.66*	*1.92 ±0.74*	*4.23 ±0.36*	*1.80 ±0.67*	*1.67 ±0.32*
Matiyal	87.5 ±2.27	86.2 ±1.39	88.6 ±1.82	83.2 ±1.93	85.8 ±1.36	97.2 ±0.86
	6.78 ±1.19	*6.54 ±1.72*	*5.68 ±1.28*	3.07 ±1.55	*5.25 ±1.45*	*9.16 ±0.96*
Matnoh	90.3 ±5.45	94.0 ±0.96	72.0 ±3.16	87.4 ±2.74	93.6 ±0.75	79.4 ±2.02
	5.63 ±0.69	*6.48 ±1.36*	*2.26 ±0.54*	*4.19 ±0.52*	*5.38 ±1.49*	*1.74 ±0.08*
Mayali	77.2 ±6.22	84.2 ±0.96	60.2 ±2.63	72.2 ±5.27	79.6 ±1.17	64.2 ±1.99
	3.78 ±0.56	11.36 ±1.66	*7.46 ±1.32*	16.27 ±0.36	43.81 ±1.42	*4.64 ±0.82*
Nagali	81.6 ±6.23	97.8 ±1.56	81.3 ±1.76	79.4 ±5.56	97.6 ±1.47	85.8 ±1.56
	7.13 ±0.98	10.62 ±1.46	*6.74 ±1.28*	*9.36 ±2.19*	10.95 ±1.29	*8.81 ±1.22*
Nihari	77.5 ±6.46	88.0 ±1.72	52.8 ±2.94	74.8 ±1.21	85.4 ±1.66	45.9 ±1.51
	6.86 ±1.54	*6.23 ±1.17*	*1.68 ±0.39*	*8.71 ±1.46*	*5.14 ±1.23*	*0.28 ±0.03*
Pabo	76.2 ±6.66	69.2 ±1.62	84.2 ±2.68	72.4 ±7.28	65.2 ±1.50	81.4 ±1.36
	5.52 ±1.28	*3.25 ±0.88*	11.08 ±1.72	*5.92 ±1.42*	*7.21 ±1.54*	13.93 ±1.09
Patwadangar	75.0 ±2.38	72.2 ±1.68	78.0 ±4.15	70.0 ±2.82	66.0 ±1.82	81.0 ±1.73
	5.98 ±0.89	*4.39 ±1.19*	*2.60 ±0.92*	*4.24 ±1.64*	*3.36 ±1.07*	*1.95 ±0.38*
Pauri	88.6 ±4.73	90.6 ±1.26	44.0 ±5.09	82.6 ±2.16	90.0 ±1.62	58.0 ±1.64
	7.25 ±1.33	10.42 ±1.21	*4.56 ±0.78*	10.71 ±0.77	17.46 ±1.17	*2.30 ±0.56*
Pokhal	96.0 ±7.48	71.4 ±2.14	86.0 ±5.10	94.0 ±2.45	65.2 ±1.40	94.0 ±1.23
	2.00 ±0.54	*4.34 ±0.76*	*5.21 ±0.82*	*3.43 ± 0.32*	*5.29 ±1.36*	*7.18 ±0.73*
Ranital	80.0 ±2.17	75.8 ±1.19	75.6 ±4.28	75.0 ±4.32	73.6 ±0.75	72.9 ±1.50
	4.62 ±1.77	*2.08 ±1.26*	*1.52 ±0.42*	*2.63 ±1.21*	*4.03 ±1.33*	*0.25 ±0.02*
Seshan	82.2 ±4.92	96.0 ±1.64	78.6 ±3.48	80.2 ±3.84	96.8 ±1.86	82.2 ±1.72
	5.92 ±1.27	*8.76 ±0.98*	*4.25 ±0.64*	*5.54 ±1.70*	*9.32 ±1.33*	*6.35 ±0.55*
Seuri	84.8 ±7.67	86.2 ±1.12	80.7 ±1.84	80.0 ±8.39	85.8 ±0.68	86.4 ±0.82
	4.34 ±0.94	*6.32 ±0.84*	*1.94 ±0.33*	*6.26 ±0.79*	*5.24 ±0.86*	*1.85 ±0.07*
Soni	90.8 ±6.59	88.4 ±1.45	80.4 ±2.91	86.6 ±4.46	86.2 ±1.69	85.2 ±1.37
	8.75 ±1.36	10.08 ±2.03	*3.21 ±1.15*	12.56 ±1.48	12.36 ±2.15	*5.05 ±1.85*
Thalisain	88.0 ±5.83	87.6 ±1.21	78.0 ±6.63	86.0 ±6.00	83.2 ±1.10	89.4 ±1.36
	2.48 ±0.40	*6.29 ±1.75*	*4.37 ±0.69*	*3.79 ±0.60*	*6.10 ±1.55*	*5.32 ±0.89*
Mean	83.04	83.20	71.84	79.22	80.38	74.98
	5.60	*6.55*	*4.39*	*6.79*	*9.06*	*4.59*
Range	62.0-96.0	66.0-98.8	44.0-88.6	56.2-94.0	60.8-98.4	45.9-97.2
	1.67-9.32	*2.08-11.36*	*1.52-11.08*	*1.94-16.27*	*1.80-43.81*	*0.25-14.79*
C.D at 1%	7.3	7.72	6.7	7.14	7.18	8.46
	1.32	*1.18*	*0.9*	*0.72*	*1.1*	*1.7*

The analysis of variance shows that there were significant differences in germination percentage and germination value of pre-chilled and unchilled seeds of all the seed sources at various temperatures and treatments (Table 4 and Table 5). The results of correlation between germination parameters and geographic factors (latitude, longitude, altitude and rainfall) were presented in Tables 6, 7, and 8. Statistically significant negative correlation was observed between latitude and various germination parameters; however significant positive correlation was recorded between longitude and germination parameters of various seed sources.

Table 4. Analysis of variance for germination percentage and germination value of different seed sources of *Pinus roxburghii*

Source of Variation	df	Germination percentage								
		Control			H_2O_2 1% v/v			GA_3 100 ppm		
		20°c	25°c	30°c	20°c	25°c	30°c	20°c	25°c	30°c
Temperature	2	1229.63	1530.39	3255.78	489.53	634.77	441.55	190.54	247.05	1444.26
Provenance	20	429.53	664.92	481.32	225.97	336.10	219.90	370.26	262.68	515.26
Error	40	123.88	133.71	162.66	34.70	115.64	129.24	57.72	142.92	118.66
Total sum of Square	62	16004.96	21707.56	22644.47	6886.36	12617.07	10450.58	10094.97	11464.28	17939.89
Probability		0.000316	0.000117	<0.00001	<0.00001	0.007824	0.04267	0.047102	0.19052	<0.00001
		0.000401	<0.00001	0.001716	<0.00001	0.002001	0.075339	<0.00001	0.05013	<0.00001

Source of Variation	*df*	Germination value								
		Control			H_2O_2 1% v/v			GA_3 100 ppm		
		20°c	25°c	30°c	20°c	25°c	30°c	20°c	25°c	30°c
Temperarure	2	795.92	7.22	10.06	86.63	547.32	9.37	56.81	692.56	104.70
Provenance	20	160.41	62.60	44.78	24.01	287.33	213.05	28.33	177.93	76.93
Error	40	51.68	31.02	2.44	7.30	49.14	11.08	5.24	44.80	24.27
Total sum of Square	62	6867.16	2507.04	1013.32	945.41	8806.88	4723.06	889.81	6735.60	2718.61
Probability		<0.00001	0.793357	0.023568	<0.00001	0.000143	0.436945	0.000173	<0.00001	0.020099
		0.001125	0.029068	<0.00001	0.000662	<0.00001	<0.00001	<0.00001	0.000101	0.00093

All parameter are significant at 5% level

Table 5. Analysis of variance for Pre-chilled and Unchilled seeds germination percentages and germination value of different seed sources of *Pinus roxburghii*

Source of variation	Degree of Freedom *df*	Germination percentages					
		Pre-chilled			Unchilled		
		20°c	25°c	30°c	20°c	25°c	30°c
Temperarure	2	345.18	467.99	1097.41	176.48	222.75	1355.78
Provenance	20	253.59	331.29	635.32	394.72	275.83	544.95
Error	40	33.32	112.16	129.66	50.17	139.85	127.11
Total	62	7094.90	12048.21	20087.50	10254.28	11555.94	18694.96
Probability		0.000237	0.022603	0.00086	0.039157	0.215972	0.000194
		<0.00001	0.001743	<0.00001	<0.00001	0.0334	<0.00001

Source of variation	Degree of Freedom *(df)*	Germination value					
		Pre-chilled			Unchilled		
		20°c	25°c	30°c	20°c	25°c	30°c
Temperarure	2	86.63	799.35	113.87	0.25	34.16	10.06
Provenance	20	24.01	134.54	55.70	45.19	165.38	46.27
Error	40	7.30	59.36	23.65	2.49	10.53	2.44
Total	62	945.41	6663.99	2287.80	1004.00	3797.14	1043.07
Probability		<0.00001	<0.00001	0.013377	0.9061	0.049479	0.023568
		0.000662	0.013647	0.010417	<0.00001	<0.00001	<0.00001

All parameter are significant at 5% level

Table 6. Correlation coefficient between germination percentages and geographic parameters of the seed sources of *Pinus roxburghii*

Geographic parameters	20°C			25°C			30°C		
	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)
Latitude	-0.2813	-0.5168	-0.2866	-0.1889	-0.5229	0.0482	-0.2605	-0.0689	-0.2467
Longitude	0.3075	0.5012	0.3196	0.1389	0.3786	-0.1395	0.3375	0.1613	0.1849
Altitude (m)	0.2830	0.0859	0.1424	0.3886	0.1680	0.2969	0.3607	0.1604	0.0868
Rainfall (mm)	-0.0433	0.1139	-0.1687	0.0437	0.2917	-0.0510	0.0753	-0.0659	0.0179

Table 7. Correlation coefficient between germination values and geographic parameters of the seed sources of *Pinus roxburghii*

Geographic parameters	20°C			25°C			30°C		
	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)	control	H_2O_2 (1%v/v)	GA_3 (100ppm)
Latitude	-0.2059	-0.5698	-0.2901	-0.2505	-0.4444	-0.3190	-0.3116	-0.2523	-0.2490
Longitude	0.0173	0.4387	0.1426	0.0836	0.3032	0.1398	0.3332	0.2842	0.2227
Altitude (m)	0.0325	0.2498	0.2930	-0.0297	0.3002	0.2803	0.2036	0.1298	0.0405
Rainfall (mm)	-0.0567	0.0723	-0.1230	-0.1530	0.0349	-0.0386	-0.0976	-0.1453	-0.1928

Table 8. Correlation coefficient between the germination percentages and germination values of Pre-chilled, Unchilled seeds and the geographic parameters of the seed sources of *Pinus roxburghii*

Geographic parameters	Germination percentages						Germination values					
	Pre-chilled			Unchilled			Pre-chilled			Unchilled		
	20°c	25°c	30°c	20°c	25°c	30°c	20°c	25°c	30°c	20°c	25°c	30°c
Latitude	-0.3181	0.0216	-0.1769	-0.2866	0.0482	-0.2467	-0.1832	-0.2046	-0.2207	-0.2901	-0.3190	-0.2490
Longitude	0.3619	-0.1148	0.1140	0.3196	-0.1395	0.1849	0.0647	0.0870	0.1825	0.1426	0.1398	0.2227
Altitude (m)	0.1923	0.2930	0.0563	0.1424	0.2969	0.0868	0.2865	0.3098	0.0488	0.2930	0.2803	0.0405
Rainfall (mm)	-0.1068	-0.0590	-0.0399	-0.1687	-0.0510	0.0179	0.1854	-0.0420	-0.1981	-0.1230	-0.0386	-0.1928

Discussion

Seed germination test in the present study demonstates that seed source variation has significant effect on seed germination percentage and germination value. Germination value varied considerably among seed sources and exhibited a random pattern. Germination value is an index of combining speed and completeness of germination, which in itself is a function of seed size and weight (Czabator 1962 and Dunlap and Barnett 1983). Seeds of Mayali, Pokhal, Pabo, Dhulcheena, and Pauri sources, which have higher vigour, took minimum time to complete germination. Significant variation in germination value among seed sources is in conformity with those found in fir and spruce (Singh and Singh 1981), *Acacia* spp. (Mathur et al 1984) and *Albizia falacataria* (Bahuguna et al 1989). Khalil (1986) was of the opinion that germination energy (vigour) and germination capacity are valid criteria for early selection of fast growing provenances.

Under favourable conditions the seeds of *P. roxburghii* germinated well (60%–80%) within 7–21 days (Thapliyal 1986). Because the seeds are non-dormant, it is often assumed that preconditioning treatments are unnecessary in this species. However, Barnett (1971) found that soaking seeds in aerated water, promotes the germination of non-dormant seeds of South Pine. Stratification or chilling under moist conditions has long been recognised as a useful method of treating seeds to improve the germinability (Outcall 1991). Heydecker and Coolbear (1977), suggested many other presowing treatments that increased germination rate.

Soaking the seeds for 24 h in a solution of H_2O_2 (1% v/v) or GA_3 (10 mg·L^{-1}) had a significant effect on average germination percentage. About 82.39% germination was revealed by the seeds treated with H_2O_2, while the mean average germination percentage of untreated (control) seeds was just 70.79%**.** Simultaneously, GA_3 and H_2O_2 treatments caused an appreciable decrease, in shortening of germination period by 8 and 10 days, respectively. Similar results were recorded by Chandra and Chauhan (1976) for *Picea smithiana* and Safiq (1980) for *Nothofagus obliqua* and *Nothofagus procera* seeds. Thus, soaking of chir-pine seeds in H_2O_2 (1% v/v) should be preferred, since it involves the saving of expenditure as compared to GA_3. The cost involved in case of H_2O_2 will be approximately half the cost of GA_3. Differences in the rate of germination from provenance to provenance have also been documented by Webb and Farmer (1968) and Wilcox (1968). The sexual reproductive efficiency that can be assessed by determining germination capacity may vary with altitude. Thus, altitudinal provenances of a species may differ not only in seed germination but also in their reproductive efficiency.

The practical implication of this study is that seeds of this commercially important tree species should be pre-treated with H_2O_2 1% v/v for 24 h to optimize germination. However, it seems that the seed source is also as important as pre-treatments, as the best germination was recorded in Thalisain, Dhulcheena, Soni, Pokhal and Matnoh provenances. Hence, seed collection should be carried out from these seed sources because seed quality has definite relations to some selective range of sources.

Responses of untreated *vs* moist chilled seeds in germination trials carried out under the same photoperiod at different constant temperatures, demonstrated the non-dormant condition of *P. roxburghii* seeds. Similar results have also been reported by Wang and Berjak (2000) for *Picea mariana.* In the the present investigation, the differences in germination were statistically significant (p=0.05) between the 15 days moist-chilled and non-chilled seeds those were subjected to different constant temperatures. In terms of the totality of germination, moist chilling for 15 days had no effect on rate and percentage of germination after being subjected to 10 mg/L Gibberellic acid treatment (Wang and Berjak 2000).

Although there was still an improvement in germination of *P. roxburghii* seeds that were moist-chilled, after a 24 h GA_3 pre-treatment, but the final germination was low, which could have otherwise the practical value. Moist-chilling is acknowledged to be an effective treatment for overcoming dormancy and improving the rate as well as the percentage of germination of dormant tree seeds (Baskin and Baskin 2001). The treatment may also facilitate germination at sub-optimal temperatures (20°C), which is particularly important for spring sowing in nurseries in temperate climates. For the shallowly-dormant seeds of Douglas-fir (*Pseudotsuga mengeiesii*), Lodgepole pine (*Pinus contorta*) and Stika spruce (*Picea sitchensis*), moist-chilling is not only a requirement to alleviate dormancy, but a prolonged period for chilling is necessary to achieve rapid and uniform germination under the low temperature in early spring (Jones and Gosling 1994; Jinks and Jones 1996). Moist-chilling for 15 days did improve the rate and percentage of germination of the non-dormant *P. roxburghii* seeds at 20°C over 21 days, but total germination was not affected at temperatures 25°C and 30°C. The effect of moist-chilling on the activation of germination in the *P. roxburghii* seeds has not been previously reported and, therefore, is a new facet in understanding of the benefits of short-term maintenance of seeds in a moistened condition at 3–5°C. The present results have emanated from the *P. roxburghii* seed lots, which are considered important for forestry practices on the basis of

their maximum germination.

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